"FORMULATION AND EVALUATION OF NANOSTRUCTURED LIPID CARRIER CONTAINING ANTICANCER DRUGS"

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1. Abstract of proposed work plan/ problem:

The aim of proposed research work is to formulate and evaluate nano-structured lipid carrier (NLC) containing potent drugs such as poorly soluble anticancer which have severe side effects on healthy organizing body. There are many conventional dosage forms available in market such as tablet, capsule, etc. But these conventional treatments are coupled with so many toxicities and side effects related with drug for e.g. bulk drug requirement to have efficacy of treatment, drug wastage due to hepatic or in vivo degradation, deposition of drug in body show unwanted toxicity. So, there is a need to culminate hindrances of conventional dosage form by formulating a targeted drug delivery such as carrier approach like liposome, niosome, phytosome, erythrocytes, microspheres, pharmacosomes, solid lipid nanoparticle, nano-structured lipid carrier, etc. Amongst these, nano-structured lipid carrier can strongly immobilize drugs and prevent the particle from coalescing by virtue of the lipid matrix. Nano-structured lipid carrier show strong potential to target drug selectively without affecting normal cell thereby minimizing drug toxicity. Nano-structured lipid carrier are beneficial to increase bioavailability being biologically inert, non immunogenic in nature. Based on literature survey, we found that number of available drug have bioavailability issue with conventional form and to work on this, it is an need to develop NLCs. Drug containing nano-structured lipid carrier will be prepared byhomogenization technique or membrane contractor technique or double emulsification method or sonication etc. Nano-structured lipid carrier formulation will be evaluated and optimized on basis of various physicochemical parameter like particle size, SEM, TEM, DSC, FTIR, Zeta potential, entrapment efficiency, drug content, in-vitro andin vivo study and stability studies.

2. Introduction:

In recent years, a significant number of new APIs developed, have a high degree of hydrophobicity which makes them poorly water soluble. This has necessitated the development of formulation strategies that can address the issue of low solubility and help to improve the rate of dissolution to enhance the bioavailability of these compounds. Since the conventional drug delivery system have wide limitations in drug delivery like low systemic availability, bulk dosing, poor drug distribution at site, in vivo degradation of drug; all ultimately provide less therapeutic efficacy. However, nanosystems are able to deliver drugs to the right place, at appropriate time, and at the right dose it is one of the most attractive areas of research in drug delivery. For the past few decades, particulate systems like liposome, niosome, nanosphere, nanocapsule and nanoparticle have been used as a physical approach to alter and improve the pharmacokinetic and pharmacodynamic properties of various types of drug molecules. Among all colloidal drug carriers nano-structured lipid carrier are gaining more popularity because of their stability, ease of preparation, for achieving reduced toxicity, for increased drug efficacy & site targeted action. They have been used in vivo to protect the drug entity in the systemic circulation, restrict access of the drug to the chosen sites and to deliver the drug at a controlled and sustained rate to the site of action. Various lipids have been used in the formulation of nano-structured lipid carrier for drug delivery research to increase therapeutic benefit, while minimizing side effects. The major goals in designing nano-structured lipid carrier as a delivery system are to control particle size, surface properties and release of pharmacologically active agents in order to achieve the site-specific action of the drug at the therapeutically optimal rate and dose regimen.

Nano-structured lipid carrier is a very useful strategy towards targeted drug delivery, particularly in immunogenic diseases like HIV, AIDS, cancer and metabolic disease such as diabetes mellitus treatment where in, the limiting factor is traditional lack of selectivity of drugs towards target site. Nanostructured lipid carrier, particularly those coated with hydrophilic polymer such as poly(ethylene glycol) (PEG) known as long circulating particles, have been used as potential drug delivery because of their ability to circulate for a prolonged period time, target a particular organ, and can able to deliver DNA, peptides, enzymes and vitamins as well as hydrophobic API. The unique nano-scale structure of nanostructured lipid carrier provides significant increases in surface area to volume ratio as well as its strong drug

immobilizing property in lipid matrix notably improve, both in-vitro and in-vivo performance, as compared to the traditional drug delivery.

Basics of NLC

Nanostructured lipid carriers (NLCs) are novel pharmaceutical formulations which are composed of physiological and biocompatible lipids, aqueous phase and surfactants. However, selection of the components and their ratios can particularly influence the final behavior of the developed formulation. Regarding lipid phase, it consists of an imperfect solid lipid matrix prepared by mixing solid and liquid lipids. There are various types of lipids that had been used in formulations of NLCs such as triglycerides, partial glycerides, fatty acids, steroids and waxes. Oils (liquid lipids) and fats contain mixtures of mono-, di- and triglycerides of fatty acids of different chain length and degree of unsaturation.

Components of NLC Drug Delivery System

Solid lipids	Stearic acid, Glyceryl monostearate, Cetyl palmitate, Glycerol behenate, Glycerol palmitostearate, etc
Liquid lipids	Oleic acid, Soy bean oil, Almond oil, Squalene, Caprylic/Capric triglycerides, Caraway essential oil, etc
Surfactants	Phospholipids: Soy lecithin, egg lecithin and phosphatidylcholine, etc Ethyleneoxide/propylene oxide copolymers: Poloxamer 188, poloxamer 182, poloxamer 407 andpoloxamine 908, etc Sorbitan ethyleneoxide/propyleneoxide copolymers: Polysorbate 20, polysorbate60 and polysorbate 80, etc Alkylaryl polyetheralcohol polymers: Tyloxapol, etc Bile salts: Sodium cholate, sodium glycolcholate, sodium tauro cholate and sodium tauro deoxycholate, etc Alcohols: Ethanol and butanol, etc

Advantages of NLC

1. Use of biodegradable and biocompatible lipids make NLCs better to incorporate both lipophilic and hydrophilic drugs.
2. Can control and target drug release at site.
3. NLCs can more strongly immobilize drugs and prevent the particle from coalescing by virtue of the lipid matrix compared to emulsions.
4. Since formulation does not contain high aqueous media NLCs show better physical stability.
5. Due to incorporation of drug into lipid matrix, In vivo as well as In vitro drug is protected till reaching site of action.
6. Higher drug loading capacity of NLCs makes it more appreciable.
7. As compared to other lipoidal nanoparticulates, drug expulsion during storage is very minute.
8. NLCs can be prepared without organic solvents.
9. Can be administered both via oral, topical and parenteral route.

3. Review of Literature and Development in the subject (Previous work done in the relevant area):

1. **Baocheng Tian et al. (2017)** investigated improved efficacy of voriconazoleby nanostructured lipid carriers.Voriconazole loaded nanostructured lipid carriers (Vrc-NLCs) were formulated for enhanced drug-delivery efficiency to C. albicans to increase the antifungal activity of Vrc and to improve the treatment of infectious Candida diseases. Vrc-NLCs were prepared by a hot-melt, high-pressure homogenization method, and size distribution, ζ-potential, morphology, drug-encapsulation efficiency, drug loading, and physical stability were characterized. The antifungal activity of Vrc-NLCs in vitro was tested during planktonic and biofilm growth in C. albicans. The mean particle size of the Vrc-NLCs was 45.62±0.53 nm, and they exhibited spheroid-like morphology, smooth surfaces, and ζ-potential of -0.69±0.03 mV. Encapsulation efficiency and drug loading of Vrc-NLCs were 75.37%±2.65% and 3.77%±0.13%, respectively. Physical stability results revealed that despite the low measured ζ-potential, the dispersion of the Vrc-NLCs was stable during their 3-week storage at 4°C. The minimum inhibitory concentration of Vrc-NLCs was identical to that of Vrc. However, the inhibition rate of Vrc-NLCs at lower concentrations was significantly higher than that of Vrc during planktonic growth in C. albicans in yeast-extract peptone dextrose medium. Surprisingly, Vrc-NLCs treatment reduced cell density in biofilm growth in C. albicans and induced more switches form hyphal cells to yeast cells compared with Vrc treatment. Study concluded, Vrc-NLCs maintain antifungal activity of Vrc and increase antifungal drug-delivery efficiency to C. albicans.

2. **Jia-You Fang et al. (2008)** developed solid lipid nanoparticles (SLN) andnanostructured lipid carriers (NLC) using Precirol ATO 5 as the solid core of the particles andPrecirol and squalene, a liquid lipid, for topical psoralen delivery respectivly. SLN and NLC showed respective mean particle sizes of 300 and 200 nm, respectively. Viscosity, polarity, and differential scanning calorimetry (DSC) studies were performed to characterize the physicochemical properties of the SLN and NLC.

The viscosity of all nanoparticulate systems exhibited Newtonian behavior except the NLC with Tween 80 and soybean phospholipids as the emulsifiers (NLC-Tw). According to the DSC thermograms, the melting peak of Precirol shifted from 58 to 55 C after incorporating squalene into the solid lipid cores of NLC, which suggests defects in the crystalline lattice of the lipid cores and smaller particle sizes. Three psoralen derivatives for psoriasis treatments were loaded in SLN and NLC to examine their ability to permeate skin. The permeability of psoralens increased in the order of 8-methoxypsoralen (8-MOP) > 5-methoxypsoralen (5-MOP) > 4,5,8-trimethylpsoralen (TMP). Enhanced permeation and controlled release of psoralen delivery were both achieved using the NLC. The in vitro permeation results showed that NLC-Tw increased the 8-MOP flux 2.8 times over that of a conventional emulsion. Hyperproliferative or psoriasis-like skin produced by repeated strippings in the dorsal skin of nude mouse was also used as a permeation barrier. The results showed that the entrapment of 8-MOP in NLC systems could minimize the permeation differentiation between normal and hyperproliferative skin compared to the free drug in an aqueous control.

3. **Xiaohong Linet al. (2007)** aqueous dispersions of SLN and NLC were successfully prepared by an oil-in-water microemulsion technique using monocaprate (MC) as the solid lipid, medium chain triglyceride (MCT) as the liquid oil and polyethylene glycol sorbitanmonooleate (Tween 80) as the emulsifier. The present work aimed to modify the inner structure of an SLN by inducing liquid oil into the system to form an NLC, and then to investigate these new carriers in detail. Several methods including dynamic light scattering (DLS), environmental scanning electron microscopy (ESEM), transmission electron microscopy (TEM), X-ray diffraction (XRD) and Fourier-transform infrared (FTIR) spectra were applied to characterize the particle size, morphology and structure of the NLC and SLN. DLS data showed that the average diameters of the SLN were ~42 nm and the diameters of the NLC ranged from ~25 to ~120 nm. All samples had polydispersity indexes about 0.27. Using ESEM and TEM, SLN and NLC were observed to be nearly spherical particles. XRD, DSC and FTIR measurements revealed that imperfect crystallization occurred in the inner core of the

NLC particles. The results suggest that nanostructured lipid carriers can be produced by this microemulsion method, and incorporation of the liquid oil into the SLN may affect their encapsulation capacity.

4. **Fu-Qiang Hu et al. (2006)** employed solvent diffusion method to produce Monostearin NLC. Monostearin (MS) and caprylic/capric triglycerides (CT) were chosen as the solid lipid and liquid lipid. Clobetasol propionate used as a model drug was incorporated into the NLC. The influences of preparation temperature and CT content on physicochemical properties of the NLC were characterized. As a result, monostearin solid lipid nanoparticles (without CT content, SLN) obtained at higher temperature (70 °C) exhibited slightly higher drug loading capacity than that of 0 °C ($P < 0.05$). In contrast, the production temperature made little effect on NLC drug loading capacity ($P > 0.05$). The improved drug loading capacity was observed for NLC and it enhanced with increasing the CT content in NLC. The results were explained by differential scanning calorimetry (DSC) measurement for NLC. The incorporation of CT to NLC led to crystal order disturbance and thus left more space to accommodate drug molecules. NLC displayed a good ability to reduce the drug expulsion in storage compared to SLN. The in vitro release behaviors of NLC were dependent on the production temperature and CT content. NLC obtained at 70 °C exhibited biphasic drug release pattern with burst release at the initial 8 h and prolonged release afterwards, whereas NLC obtained at 0 °C showed basically sustained drug release throughout the release time. The drug release rates were increased with increasing the CT content. Study indicated that the NLC produced by solvent diffusion method could potentially be exploited as a carrier with improved drug loading capacity and controlled drug release.

5. **Fu-Qiang Hu et al. (2005)** prepared stearic acid (SA) nanostuctured lipid carriers with various oleic acid (OA) content by solvent diffusion method in an aqueous system. The size and surface morphology of nanoparticles were significantly influenced by OA content. As OA content increased up to 30 wt%, the obtained particles showed pronounced smaller size and more regular morphology in spherical

shape with smooth surface. Compared with solid lipid nanoparticles (SLN), NLC exhibited improved drug loading capacity, and the drug loading capacity increased with increasing OA content. These results were explained by differential scanning calorimetry (DSC) investigations. The addition of OA to nanoparticles formulation resulted in massive crystal order disturbance and less ordered matrix of NLC, and hence, increased the drug loading capacity. The drug in vitro release behavior from NLC displayed biphasic drug release pattern with burst release at the initial stage and prolonged release afterwards, and the successful control of release rate at the initial stage can be achieved by controlling OA content.

6. **R. H. Muller et al. (2002)**produced NLC by mixing solid lipids with spatially incompatible lipids leading to form special structures of the lipid matrix, i.e. three types of NLC: (I) the imperfect structured type, (II) the structureless type and (III) the multiple type. Study described a special preparation process of NLC allows the production of highly concentrated particle dispersions (30–95%) with potential applications as drug delivery system.

4. Objectives of Research/ Proposed Hypothesis:

Aim: Formulation and Evaluation of Nanostructured Lipid Carrier Containing Anticancer Drugs.

Objectives of the Research Work:

- To formulate, optimize and characterizeanticancer drug-loaded nanostructured lipid carrier drug delivery system.
- To improve the efficacy, drug loading capacity and biocompatibility of poorly solubleanticancerdrug by formulating in nanostructured lipid carrier.
- To minimize the toxicity and increase the stability of poorly solubleanticancerdrug by encapsulating in nanostructured lipid carrier system.
- To study In-vivo anticancer activity of end product.
- To evaluate in-vivo bioavailability of drug from formulation in comparison with pure drug in experimental animal.
- To study the tissue distribution of drug from formulation in comparison with pure drug in experimental animal.

5. Materials and Methodology to be adopted:

1. **Literature review**
2. **Selection and Collection of drug & excipients**

   Selection of drug: e.g. Anticancer drug.

   Selection of a lipid: To formulate the Nanostructured Lipid Carrier, following solid and liquid lipids will be selected e.g. Soybean oil or Oleic acid or Corn oil or Squaleneor Stearic acid etc.
3. **Experimental work**
   - **A. Preformulation Studies**
     1. **Spectroscopic study**
     2. **Solubility study**
     3. **Crystallinity study**
   - **B. Compatibility studies**
4. **Formulation development**
   - Preparation of Nanostructured Lipid Carrier by suitable method.
   - Lyophilization of drug-loaded Nanostructured Lipid Carrier
5. **Characterization of drug-loaded Nanostructured Lipid Carrier**
   - Yield and drug content
   - Particle size and zeta potential
   - Encapsulation efficiency
   - Surface morphology by Transmission electron microscopy (TEM)
   - Fourier transform infrared spectroscopy
   - XRD study
   - Differential scanning colorimetry
   - In vitro drug release
6. **Stability studies**
7. **In vitro anticancer study**
8. **In vivo Pharmacokinetic study**
9. **In vivo Anticancer study**

6. Importance of study/Society application:

- **International status:** Globally a nanodrug delivery is an emerging area for novel applications in oral, topical and parenteral drug delivery. In combination with the evolution of its applications in large domains, nanoscience has been contributing its remarkable role in drug delivery too. Recently, the global pharma world has witnessed marvelous development in introducing innovative nanotechnologics, exploring their applications in temporal and spatial drug delivery, addressing their toxicity concerns and resolving key federal issues. Nanostructured lipid carrier drug delivery systems, accordingly arc gaining widespread importance and popularity globally. There are potential applications of nanosystems in drug delivery or gene delivery areas like delivering small particles in the tumours, across the blood- brain barrier and other sites through diverse routes of drug administration.

  US patent application 20170119891: Nano-structured carriers for guided and targeted on-demand substance delivery (Granted on 04-05-2017).

  South Korean patent application KR101777616B1: Nano-structured Lipid Carrier comprising α-tocopherol (Granted on 13-09-2017).

- **National status:** India being ranked second largest populated country in the world, facing a many challenges in immunogenic diseases like HIV, AIDS, cancer and metabolic disease such as diabetes mellitus therapy. Number of patients in India is increasing due to sedentary life style, poor living standard (diet & rest), pollution, lack of awareness early signs and symptoms etc. A big issue in India is lack of availability of most effective, safe and economic drug delivery for treatment of diseases either topical or systemic occurance. Nanoparticulates, especially nano-structured lipid carrier have the potential to overcome the challenges related to conventional treatments. There is huge scope in India to develop a drug delivery with improved efficacy, biocompatibility, biodistribution and minimized toxicity in drug therapy.

►**Significance of the study:**

- Nanotechnology is becoming the driving force behind a variety of evolutionary and revolutionary changes in the medical field globally. The impact of nanotechnology on drug delivery has helped to improve the efficacy of drug therapeutics and will likely enable the creation of entirely new therapeutic entities. Future of nanomedicine, given the wide array of innovative nanoscale materials and technologies that stand on the horizon. With the clarification of nanotechnology-specific medical regulations and a continued influx of investments and time, we believe that nanomedicine will not only improve conventional therapies, but also bridge the shortcomings of conventional medicine to help people on both global and individual levels.
- Currently, large proportion of solid active pharmaceutical ingredients (API) has poor aqueous solubility, and therefore poor bioavailability. Conventional drug delivery system of such poorly soluble drugs have wide limitations in drug delivery like low systemic availability, bulk dosing, poor drug distribution at site, in vivo degradation of drug; all ultimately provide less therapeutic efficacy. So, to overcome these challenges it becomes necessary to formulate a novel drug delivery system which is more promising, more effective and safe.
- Nanostructured lipid carrier provides large influences that are a prerequisite in formulating a stable drug delivery system. NLCs are chemically and physically stable system with improved drug incorporation and increased bioavailability. The increasing interest of industry in lipid carrier system has been remarkably expanding the advancements in latest years.

7. Proposed work Plan/ Formulation and Structure of Study:

Year-wise Plan of work and targets to be achieved:

Sr. No.	Expected target to be achieved	1-6 months	7-10 months	11-22months	22-27 months	28-33 months	34-36 months
01	Literature survey	Literature survey					
02	Selection and collection of drug and excipients	Selection and collection of drug and excipients					
03	Formulation of nanostructured lipid carriers	Formulation of nanostructured lipid carriers					
04	Evaluation of nanostructured lipid carriers	Evaluation of nanostructured lipid carriers					
05	In vitro and in vivo study	In vitro and in vivo study					
06	Statistical analysis, data interpretation and conclusions	Statistical analysis, data interpretation and conclusions					
07	Publishing research and submitting data	Publishing research and submitting data					

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